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Frank Laboratory of Neutron Physics

FINAL REPORT ON THE START PROGRAMME

Experimental and in silico investigation of ketoprofen

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ABSTRACT

The report presents the results of research work carried out during JINR student programme “START” and deals with experimental as well as computational investigations of ketoprofen. Ketoprofen is a representative of the group of non-steroidal anti-inflammatory drugs widely used in modern medical therapy. It has a high analgesic, anti-inflammatory and antipyretic activity. Reactivity of ketoprofen is closely connected with is structures features. Ketoprofen sample was investigated by the following experimental methods: differential scanning calorimetry, X-ray powder diffractometry and IR-spectroscopy. The structure of ketoprofen was also investigated by DFT (BP86/def2-SVP) and semi-empirical (PM6) methods. A comparative analysis of the calculated and experimental data was performed. Investigation of ketoprofen sample by IR spectroscopy demonstrated concordance of spectra obtained in KBr tablet and by FTIR method. The most intensive peaks in these spectra corresponds to the ν(C-O) and two ν(C=O) vibrations. A good agreement between the experimental and calculated in the DFT approximation vibrational frequencies of ketoprofen was obtained. Linear correlation was also found between the values calculated by the PM6 method and the DFT method. The BP86/def2-SVP level of theory can be recommended for further *in silico* structural studies of ketoprofen.

1. INTRODUCTION

Ketoprofen is a representative of the group of non-steroidal anti-inflammatory drugs widely used in modern medical therapy. It has a high analgesic, anti-inflammatory and antipyretic activity. Ketoprofen is a racemic mixture of enantiomers in which the pharmacological activity is associated with the S-enantiomer; the R-isomer has the ability to inhibit cyclooxygenase is much lower.

Purpose of the study is the complex investigation of ketoprofen by experimental and quantum-chemical methods in order to demonstrate the sample quality and choose the characteristic parameters that can be used for the following investigations.

Object of research is a ketoprofen sample. Subject of research is key structural parameters that characterize the ketoprofen properties in the condensed matter.

The main tasks of the study are:

- characterization of ketoprofen by DSC method;
- determination of the main characteristics of ketoprofen by powder X-ray diffraction;
- investigation of ketoprofen sample by FTIR spectroscopy;
- *in silico* calculations of vibrational frequencies of ketoprofen enantiomers by semiempirical quntum-chemical and DFT methods;
- joint analysis of the results of experimental studies and molecular modeling.

2. EXPERIMENTAL

2.1. Materials and samples

The molecular formula of ketoprofen is $C_{16}H_{14}O_3$, the chemical name is 2-(3-benzoylphenyl) propionic acid [1]. The molecular weight is 254.28 g/mol. Ketoprofen is an odorless white powder. It is easily soluble in alcohol, chloroform, acetone, ether, benzene and strong alkalis, but practically insoluble in water. CAS Number is 22071-15-4. The chemical structures of ketoprofen is presented in Fig. 1 [1].

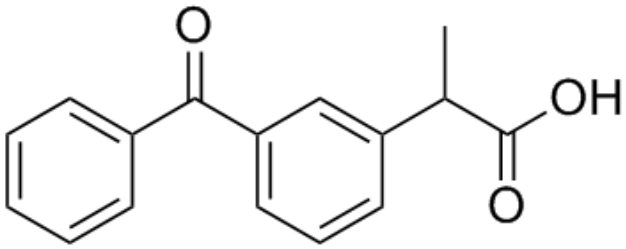
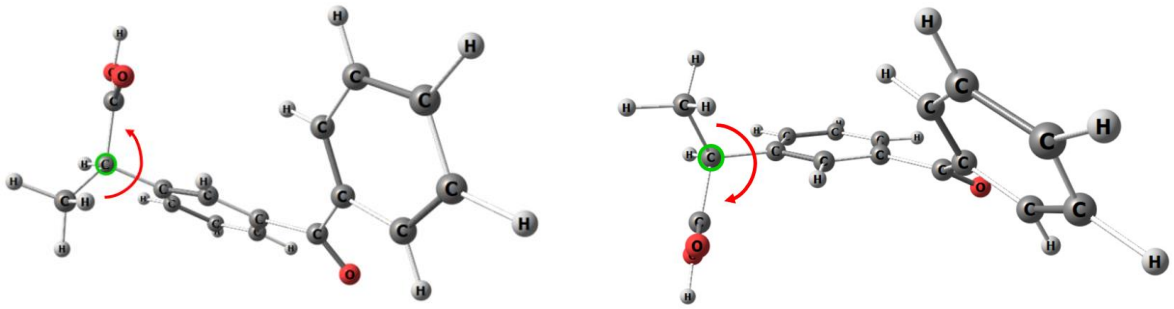


Fig. 1. Chemical structure of ketoprofen [1]

The spatial structure of the S(-) and R(+)-enantiomers of ketoprofen is shown in Fig. 2.



a) b)

Fig. 2. 3D structure of S(-)-ketoprofen and R(+)-ketoprofen under letters (a) and (b) respectively

2.2. Methods

2.2.1. Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. It provides valuable information about the thermal behavior of the sample, such as phase transitions, crystallization, melting, glass transitions, and chemical reactions. DSC can also determine the enthalpy changes associated with these thermal events, which are useful for understanding the stability, purity, and composition of materials.

DSC experiments were carried out with a Netzsch differential scanning calorimeter, model DSC-204 F1 Phoenix with a heating rate of 10 °C/min. A small amount of ketoprofen sample 3,8 mg was enclosed in a hermetic aluminum pan. The measurements were carried out in an argon atmosphere. Heating was carried out up to 120 °C.

2.2.2. X-ray powder diffractometry

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. This method involves the emission of X-rays onto a powdered sample and the analysis of the resulting diffraction pattern. When X-rays interact with a crystalline material, they diffract due to the correct arrangement of atoms in the crystal lattice.

X-ray diffraction analysis of the powder sample of ketoprofen was done at room temperature using a type PAN Analythical Diffractometer EMPYREAN. The measurement condition is described as follow: Co metal target, Kα filter, voltage 40 kV, 40 mA current, the analysis performed on the 2 theta range 3-50°. The sample was placed on the sample holder and leveled to prevent particle disorientation during sample preparation. The cuvette is made of Si monocrystal.

2.2.3. Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) is a technique used to analyze the chemical composition and structure of a substance based on its interaction with infrared light. The infrared spectrum of a sample is obtained by passing infrared light through it and measuring the amount of light absorbed at different wavelengths.

Infrared transmission spectrum of ketoprofen in KBr disks (ca. 0,3 %, w/w) were recorded at room temperature on a Nicolet iS50 FTIR spectrometer, in the range 400–4000 cm^{-1}. IR reflection spectrum of disturbed total internal reflection were measured at room temperature on a spectrometer Nicolet iS5 FTIR in a frequency interval of 400–4000 cm^{-1}.

2.2.3. Computational details

Calculations optimization of geometric parameters and calculation of IR spectra for enantiomers R and S were performed using the software package ORCA [2] in the framework of the density functional theory (DFT) method using the BP86 functional and def2-SVP base. BP86 functional consisting of Becke 88 gradient corrections for the exchange functional and the Perdew 86 correlation expression [3].

Additional research was carried out using a semi-empirical method in the PM6 [4] approximation in the MOPAC2016 [5] program. The PM6 method was developed by Stewart in 2007. This semi-empirical method follows the traditions of other NDDO methods, such as MNDO, AM1, PM3. "PM" from the name of the method means "parameterization of the method". Some errors in the AM1 and PM3 methods were corrected during the development of the PM6 method.

3. RESULTS AND DISCUSSION

3.1. Thermal behaviour of ketoprofen

A sample of ketoprofen was examined for crystallinity by DSC. The experimental DSC curve of ketoprofen sample is shown in Fig. 3. The sample was heated from 24 ˚C up to 120 ˚C and after that was cooled down to the 24 ˚C. The curve obtained as a result of heating (curve 1.2 in Fig. 3) contains a peak corresponding to the melting point of ketoprofen. No extrema were observed on the cooling curve (line 1.3 in Fig. 3) in the studied range.

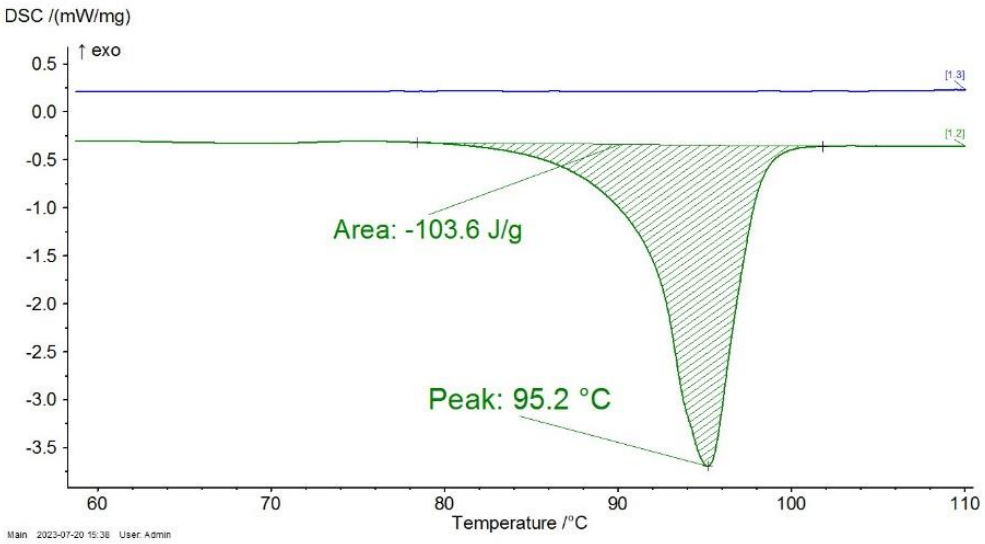


Fig. 3. DSC results of ketoprofen sample

Experimentally obtained melting point of ketoprofen ($T_{peak\ fusion}$ = 95.2 ˚C) were compared with that form the NIST Database from the article [6]. This value are in a good agreement with the value of $T_{peak\ fusion}$ = 96˚C presented in the database [6].

3.2. XRPD of ketoprofen

X-ray diffraction pattern of ketoprofen sample presented on Fig. 4. contains sharp diffraction peaks indicated that ketoprofen is in a crystalline form. The interplanar distances based on the values of 2Theta were calculated using the Wulff-Bragg's equation and are listed in Table 1. The interplanar distances for ketoprofen taken from reference [7] are also listed. The positions of the main peaks of the

diffraction pattern were compared with those presented in the card for ketoprofen in the ICDD PDF-4 database (reference code 00-038-1582). The data presented in the card are taken from the reference [7]. The obtained values of interplanar spacings and relative peak intensities for studied ketoprofen sample are in good agreement with the values from the article [7].

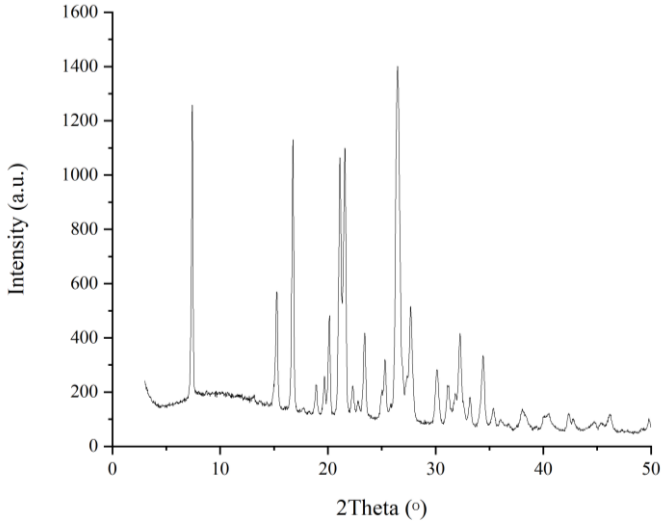


Fig. 4. X-ray diffractogram of ketoprofen sample

Table 1. Values of interplanar spacings and relative intensities of lines for ketoprofen

$2Theta_{exp}$ (°)	Peak max, %	d_{exp}, Å	d_{ref} [7], Å
7.4	89.8	13.86	13.80
15.2	**40.7**	6.76	6.76
16.8	**80.7**	6.12	6.19
18.9	16.0	5.45	5.47
19.7	18.1	5.23	5.25
20.2	34.4	5.10	5.13
21.1	**75.9**	4.88	4.85
21.6	**78.5**	4.77	4.62
22.3	15.5	4.63	4.44
22.8	11.9	4.53	4.04
23.4	29.9	4.41	3.90
25.3	22.4	4.08	3.74
26.5	**100.0**	3.90	3.43
27.7	36.6	3.74	3.29
30.1	20.2	3.44	3.23
31.1	16.1	3.34	3.19
32.3	29.7	3.22	3.14
33.2	12.7	3.13	3.04
34.4	23.6	3.02	2.74
35.4	9.9	2.94	2.60

It was proposed by authors [7] that for identification purposes it is enough that the three most intense lines coincide. Some variation between the intensities of the strongest lines in the diffraction patterns obtained are explained in terms of preferred orientation in the specimens, as is pointed out in ref. [7, 8].

Thus, obtained results for ketoprofen sample analysis by x-ray powder diffractometry correspond to the available experimental data.

3.3. Vibrational analysis of ketoprofen

Experimental studies of ketoprofen by IR-spectroscopy were performed in a dispersion of potassium bromide and by the spectroscopy of disturbed total internal reflection. The resulting spectra are concordant and are shown in Fig. 5 along with labeled peaks and corresponding oscillation types within 400–4000 cm^{-1}. The insignificant difference in the observed peak intensities is due to the experimental conditions and the features of the methods used.

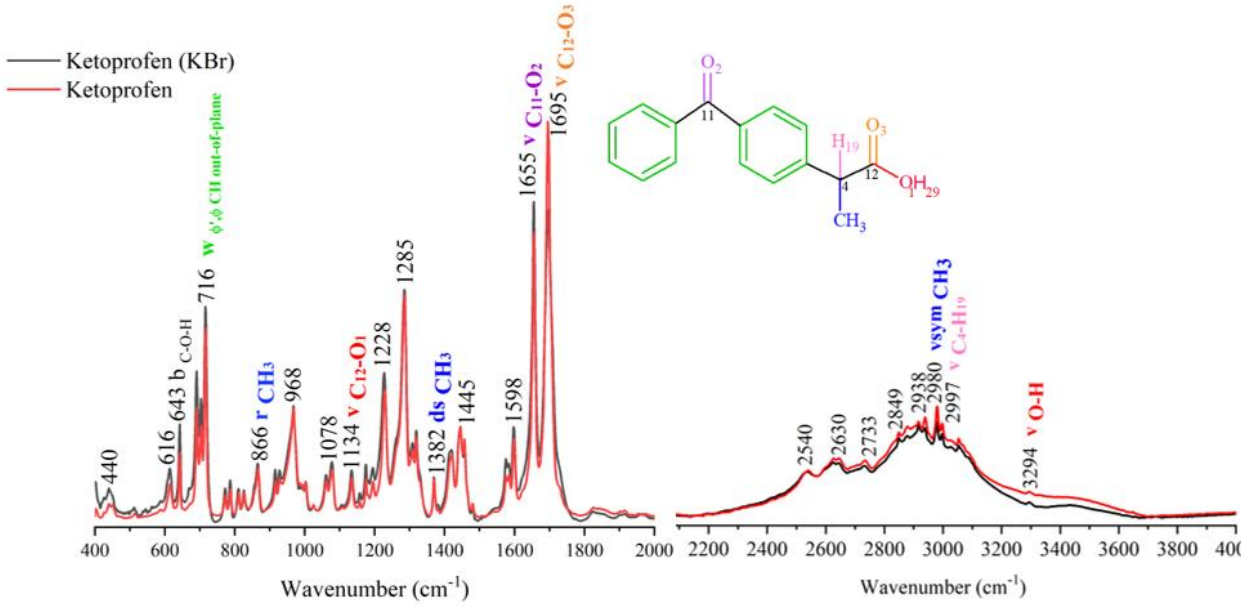


Fig. 5. FTIR experimental spectra (400–4000 cm^{-1}) of ketoprofen sample (ν – stretching, ν_{sym} – symmetric stretching, w – wagging deformation, ds – symmetric deformation)

Experimental FTIR wavenumbers for the main peaks of ketoprofen are presented in Table 2, along with those ones calculated by DFT method for the two most stable conformers of R- and S-enantiomers of ketoprofen (Fig. 2).

Table 2. Experimental FTIR and DFT calculated harmonic wavenumbers (cm^{-1}) for ketoprofen

FTIR	DFT (BP86/def2-SVP)		Approximate descriptions
	Enantiomer R	Enantiomer S	
716	714	713	ϕ', ϕ CH out-of-plane wagging
866	844	843	C-O-H bending, Φ' CH out-of-plane bending, CH_3 rocking
1134	1134	1132	ϕ', ϕ in-plane bending, CH_3 rocking,C_{12}-O_1 stretching, O_1-H_{29} bending
1382	1410	1410	CH_3 symmetric deformation, C_4-H_{19} stretching, Φ' in-plane bending
1655	1686	1684	C_{11}=O_2 stretching
1695	1785	1784	C_{12}=O_3 stretching
2980	2973	2974	CH_3 symmetric stretching
2997	3002	3005	C_4-H_{19} stretching
3294			OH stretching (H-bonded)
	3604	3602	OH stretching (free)

The obtained experimental frequencies of vibrations of the main groups of ketoprofen can be used to assess the accuracy of the calculated values obtained as a result of molecular modeling.

3.4. Semi-empirical and DFT calculations

3.4.1. DFT calculations

At the first stage, the optimization of the molecular geometry of the R- and S-enantiomers of ketoprofen was performed using the BP86 hybrid functional and the def2-SVP basis set. Based on the obtained equilibrium configurations (see Fig. 2), the harmonic vibrational frequencies were calculated. It can be inferred that the DFT-calculated frequencies for studied enantiomers are not differ essentially (Table 2). Fig. 6 displays the DFT-calculated IR-spectrum of the S-enantiomer (similar spectrum can be observed for the R enantiomer) with labeled main peaks. Visualization of calculated IR-spectrum was performed using ChemCraft software [9].

It can be seen from the spectrum that the main peaks take values 1135, 1689 and 1783 cm^{-1}, which corresponded to motions of v(C-O) and two v(C=O)

respectively. The peak that corresponds to stretching of the O-H bond (3294 cm^{-1}) is shifted to the lower wavenumbers as compared to the calculated one. Experimental value corresponds to the H-bonded group vibration, while calculated value corresponds to the free O-H bond stretching. Experimental vs calculated values of wavenumbers for corresponded IR-spectra of ketoprofen are presented in the Fig. 7.

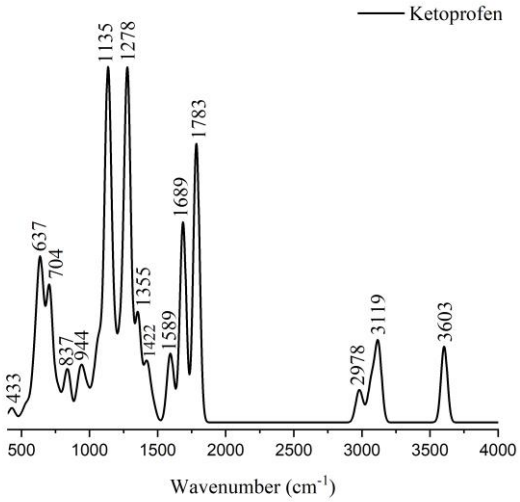


Fig. 6. Calculated IR-spectrum (400–4000 cm^{-1}) of the S-enantiomer of ketoprofen (BP86/def2-SVP)

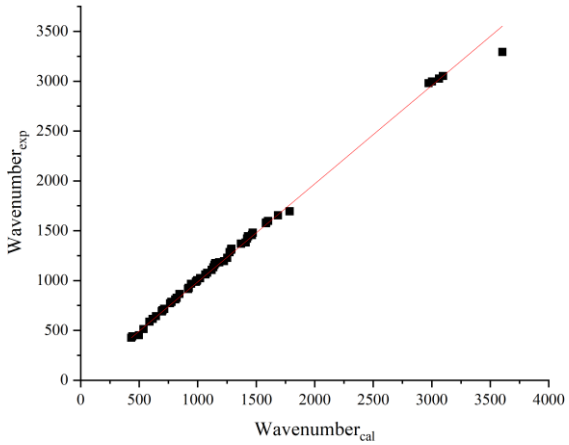


Fig. 7. Experimental vs DFT-calculated values of wavenumbers for ketoprofen

There is a good agreement between the experimental and calculated in the DFT approximation vibrational frequencies of ketoprofen. Straight line on Fig. 7 is described by equations (1). The slope value obtained is in a good agreement with scale factors for DFT-methods [10].

$$\nu_{exp} = (0{,}99 \pm 0{,}00) \cdot \nu_{cal} \ (R = 0{,}99953) \quad (1)$$

Thus, the BP86/def2-SVP level of theory can be recommended for further *in silico* structural studies of ketoprofen.

3.4.2. Semi-empirical calculations

Conformational mobility can greatly affect the reactivity and biological activity of organic compounds. In the structure of ketoprofen, one of the possible reaction center is the carboxyl group. To study the intramolecular dynamics of the carboxyl group. Additional calculations by semi-empirical PM6 method were carried out to investigate the internal dynamics of carboxyl group of ketoprofen. The optimization of the molecular geometry of the R- and S-enantiomers of ketoprofen was performed following by the harmonic vibrational frequencies calculations.

A linear correlation (R = 0,9966) is obtained between the values of vibrational frequencies calculated in the approximation of the PM6 method and the DFT method at the BP86/def2-SVP level of theory (Fig. 8).

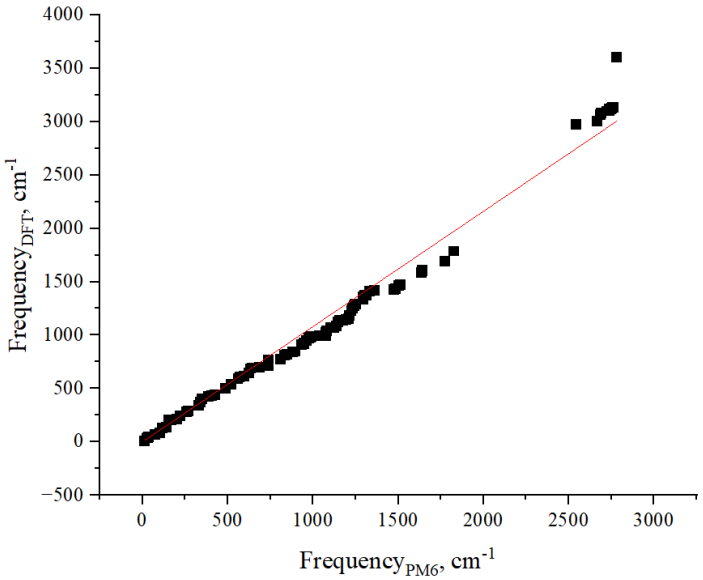


Fig. 8. Linear correlation between values obtained by the DFT and PM6 methods for ketoprofen

The straight line on Fig. 8 is described by equations (2).

$$\nu_{DFT} = (1{,}07974 \pm 0{,}00931) \cdot \nu_{PM6} \ (R = 0{,}9966) \quad (2)$$

A good correlation is observed for calculated values of frequencies by PM6 and DFT.

For further study the intramolecular dynamics of the carboxyl group of ibuprofen enantiomers, the torsion angle $O_3C_{12}C_4C_5$ was chosen as internal rotation coordinate (Fig. 9).

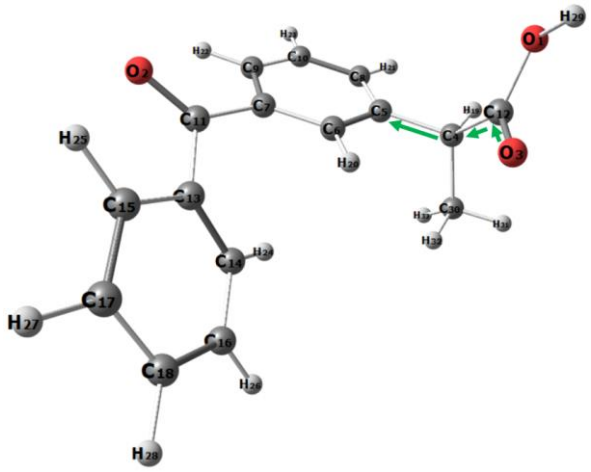
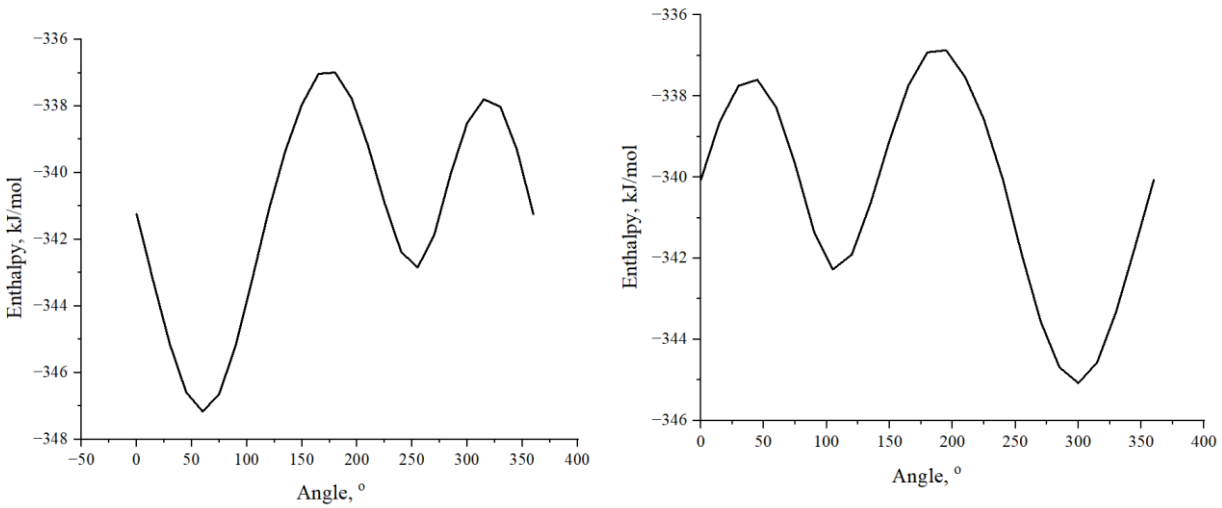


Fig. 9. Schematic representation of the R-enantiomer structure for ketoprofen with a selecting dihedral angle

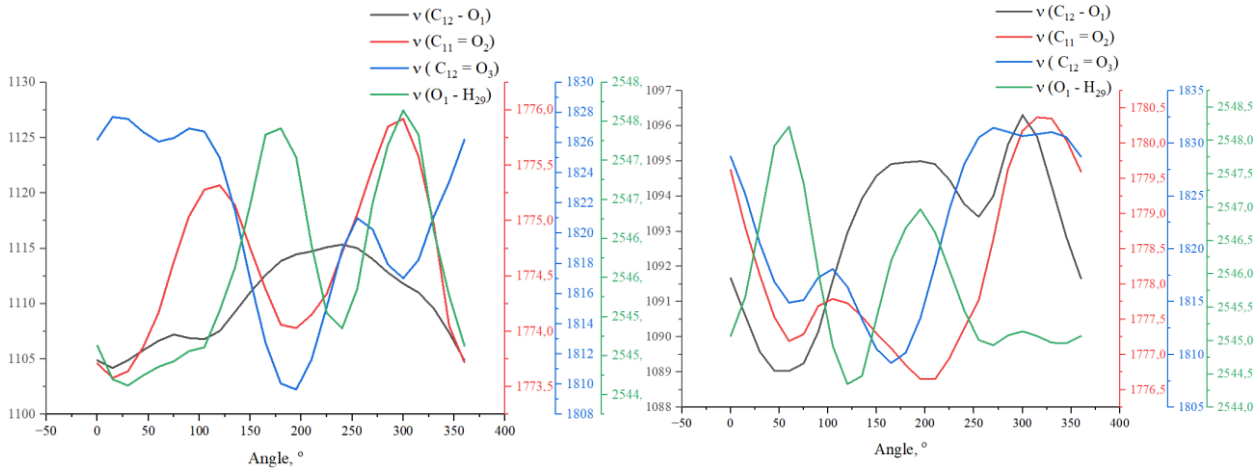
The change in the molecule enthalpy of formation during rotation of the carboxyl group with a step of 15° for two enantiomers of ketoprofen is shown in the Fig. 10. The profiles of changes in the formation enthalpy of ketoprofen formation during intramolecular rotation of the carboxyl group for two enantiomers have the similar character, but are mirrored. The most stable configurations correspond to different coordinates (60° and 300°). The barriers of intramolecular rotation do not exceed 10 kcal/mol, which indicates the high mobility of the studied carboxyl group of ketoprofen.



a b

Fig. 10. Plot of formation enthalpy changing along the coordinate of intramolecular rotation for R-ketoprofen (a) and S-ketoprofen (b) (PM6 calculations)

Vibrational frequencies were calculated for each coordinate of the intramolecular rotation of the carboxyl group. Fig. 11 illustrates character of the change in the frequency of stretching vibrations of the bonds for the C-O, C=O and O-H carboxyl group during its rotation around the C-C bond.



a b

Fig. 11. Plot of the main frequencies changing along the coordinate of intramolecular rotation for R-ketoprofen (a) and S-ketoprofen (b) (PM6 calculations)

The obtained profiles allow to estimate the range of variation of indicated vibrational frequencies for two enantiomers of ketoprofen. Obtained values are ~11 cm^{-1}, ~3 cm^{-1}, ~18 cm^{-1}, and ~4 cm^{-1} for v(C-O), two v(C=O) and v(C-O) and v(O-H) vibrations respectively. A noticeable changes are observed for the C=O group.

Thus, the semi-empirical PM6 method is suitable for a preliminary estimation of the conformational mobility of ketoprofen enantiomers and the character of changes in the parameters of its IR spectrum.

CONCLUSION

Main characteristics of investigated ketoprofen sample determined by experimental methods correspond to those given in the literature.

It was demonstrated by powder X-ray diffraction method that investigated ketoprofen sample is in a crystalline state. The obtained diffraction pattern corresponds to the data given in the literature. A joint analysis of the obtained experimental and literature data showed that the signal observed at the value of $2\theta = 7{,}4°$ cannot be used to ketoprofen identification, since its intensity depends on the orientation of the crystallites during sample preparation.

The melting point (95.2 °C) of ketoprofen sample was determined using differential scanning calorimetry, and these values are in agreement with the data reported in the literature.

Investigation of ketoprofen sample by IR spectroscopy demonstrated concordance of spectra obtained in KBr tablet and by FTIR method. The most intensive peaks in these spectra corresponds to the ν(C-O) and two ν(C=O) vibrations. There is a good agreement between the experimental and calculated in the DFT approximation vibrational frequencies of ketoprofen. Linear correlations are obtained between the experimental vibrational frequencies and the values calculated by the DFT method. The BP86 and def2-SVP level of theory can be recommended for further *in silico* structural studies of ketoprofen.

According to the results of *in silico* studies of ketoprofen enantiomers, no significant differences were found in their IR spectra.

In silico study of the intramolecular dynamics of the carboxyl group of ketoprofen using the PM6 method showed that when the orientation of the -COOH group changes, the positions of its key vibrational modes in the IR spectrum change within 1655 and 1695 cm^{-1}.

For a more accurate and complete description of the experimental vibrational spectra, additional *in silico* studies of the molecular dynamics of ketoprofen in the fused state are required.

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